Cell, Volume *169*

Supplemental Information

Recurrent Potent Human Neutralizing Antibodies to Zika Virus in Brazil and Mexico

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Supplemental Tables

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Table S1. V(D)J rearrangements and sequences of anti-ZEDIII antibodies that do cluster in clones. Excel file. Related to Figure 2.

Table S2. V(D)J rearrangements and CDR3 sequences of anti-ZEDIII antibodies that do not cluster in clones (singlets). Excel file. Related to Figure 2.

Table S3. List of primers for cloning recombinant antibodies by the SLIC method. Related to Methods.

Table S4. List of primers for the generation of RVP expression constructs. Related to Methods.

Table S5. Data collection and refinement statistics. Related to Figure 5.

Table S6. Antibody-antigen contacts. Related to Figure 5.

Table S3. List of primers for cloning recombinant antibodies by the SLIC method. Related to Methods.

Primer ID	Primer sequence	Best V or J gene segment match
Human antibody heavy chain (forward)		
p1355DFR	5'CTAGTAGCAACTGCAACCGGTGTACATTCCCAGGTGCAGCTGGTGCAG	VH 1
p1356DFR	5'CTAGTAGCAACTGCAACCGGTGTACATTCCGAGGTGCAGCTGGTGCAG	VH 1/5
p1357DFR	5'CTAGTAGCAACTGCAACCGGTGTACATTCCCAGGTTCAGCTGGTGCAG	VH 1-18
p1358DFR	5'CTAGTAGCAACTGCAACCGGTGTACATTCCCAGGTCCAGCTGGTACAG	VH 1-24
p1359DFR	5'CTAGTAGCAACTGCAACCGGTGTACATTCTGAGGTGCAGCTGGTGGAG	VH 3
p1360DFR	5'CTAGTAGCAACTGCAACCGGTGTACATTCTCAGGTGCAGCTGGTGGAG	VH 3-11
p1361DFR	5'CTAGTAGCAACTGCAACCGGTGTACATTCTGAGGTGCAGCTGTTGGAG	VH 3-23
p1362DFR	5'CTAGTAGCAACTGCAACCGGTGTACATTCTCAGGTGCAGCTGGTGGAG	VH 3-33
p1363DFR	5'CTAGTAGCAACTGCAACCGGTGTACATTCTGAAGTGCAGCTGGTGGAG	VH 3-9
p1364DFR	5'CTAGTAGCAACTGCAACCGGTGTACATTCCCAGGTGCAGCTGCAGGAG	VH 4
p1365DFR	5'CTAGTAGCAACTGCAACCGGTGTACATTCCCAGGTGCAGCTACAGCAGTG	VH 4-34
p1366DFR	5'CTAGTAGCAACTGCAACCGGTGTACATTCCCAGCTGCAGCTGCAGGAG	VH 4-39
p1367DFR	5'CTAGTAGCAACTGCAACCGGTGTACATTCCCAGGTACAGCTGCAGCAG	VH 6-1
Human antibody heavy chain (reverse)		
p1370DFR	5'CCGATGGGCCCTTGGTCGACGCTGAGGAGACGGTGACCAG	JH 1/2
p1371DFR	5'CCGATGGGCCCTTGGTCGACGCTGAAGAGACGGTGACCATTG	JH 3
p1372DFR	5'CCGATGGGCCCTTGGTCGACGCTGAGGAGACGGTGACCAG	JH 4/5
p1373DFR	5'CCGATGGGCCCTTGGTCGACGCTGAGGAGACGGTGACCGTG	JH 6
Human antibody light chain kappa (forward)		
p1379DFR	5'GTAGCAACTGCAACCGGTGTACATTCTGACATCCAGATGACCCAGTC	VK 1-5
p1380DFR	5'GTAGCAACTGCAACCGGTGTACATTCAGACATCCAGTTGACCCAGTCT	VK 1-9
p1381DFR	5'GTAGCAACTGCAACCGGTGTACATTGTGCCATCCGGATGACCCAGTC	VK 1D-43
p1382DFR	5'GTAGCAACTGCAACCGGTGTACATGGGGATATTGTGATGACCCAGAC	VK 2-2
p1383DFR	5'GTAGCAACTGCAACCGGTGTACATGGGGATATTGTGATGACTCAGTC	VK 2-28
p1384DFR	5'GTAGCAACTGCAACCGGTGTACATGGGGATGTTGTGATGACTCAGTC	VK 2-30
p1385DFR	5'GTAGCAACTGCAACCGGTGTACATTCAGAAATTGTGTTGACACAGTC	VK 3-11
p1386DFR	5'GTAGCAACTGCAACCGGTGTACATTCAGAAATAGTGATGACGCAGTC	VK 3-15
p1387DFR	5'GTAGCAACTGCAACCGGTGTACATTCAGAAATTGTGTTGACGCAGTCT	VK 3-20
p1388DFR	5'GTAGCAACTGCAACCGGTGTACATTCGGACATCGTGATGACCCAGTC	VK 4-1
Human antibody light chain kappa (reverse)		
p1390DFR	GAAGACAGATGGTGCAGCCACCGTACGTTTGATYTCCACCTTGGTC	JK 1/4
p1391DFR	GAAGACAGATGGTGCAGCCACCGTACGTTTGATCTCCAGCTTGGTC	JK 2
p1392DFR	GAAGACAGATGGTGCAGCCACCGTACGTTTGATATCCACTTTGGTC	JK 3
p1393DFR	GAAGACAGATGGTGCAGCCACCGTACGTTTAATCTCCAGTCGTGTC	JK 5
Human antibody light chain lambda (forward)		
p1402DFR	CTAGTAGCAACTGCAACCGGTTCCTGGGCCCAGTCTGTGCTGACKCAG	VL 1
p1403DFR	CTAGTAGCAACTGCAACCGGTTCCTGGGCCCAGTCTGCCCTGACTCAG	VL 2
p1404DFR	CTAGTAGCAACTGCAACCGGTTCTGTGACCTCCTATGAGCTGACWCAG	VL 3
p1405DFR	CTAGTAGCAACTGCAACCGGTTCTCTCTCSCAGCYTGTGCTGACTCA	VL 4/5
p1406DFR	CTAGTAGCAACTGCAACCGGTTCTTGGGCCAATTTTATGCTGACTCAG	VL 6
p1407DFR	CTAGTAGCAACTGCAACCGGTTCCAATTCYCAGRCTGTGGTGACYCAG	VL 7/8
Human antibody light chain lambda (reverse)		
p1409DFR	GGCTTGAAGCTCCTCACTCGAGGGYGGGAACAGAGTG	Constant L

Table S4. List of primers for the generation of RVP expression constructs. Related to Methods.

Primer ID	Primer sequence	Comments
Mutation of BspHI sites in pZIKV/HPF/CprME to generate pZIKV/HPF/CprM*E*		
RU-O-24303	GTTAAGGGATTTTGGACATGAGATTATC	BspHI at nt 6419 forward
RU-O-24304	GATAATCTCATGTCCAAAATCCCTTAAC	BspHI at nt 6419 reverse
RU-O-24309	CTTGGTTGAGTACTCACCAGTCA	Reverse outer (for 6419)
RU-O-24310	GAAGACTACAGCGTCGCCAG	Forward outer (for 6419)
RU-O-24305	GTTATTGTCTCATGCGCGGATAC	BspHI at nt 7427 forward
RU-O-24306	GTATCCGCGCATGAGACAATAAC	BspHI at nt 7427 reverse
RU-O-24307	CTTCATGCAATTGTCGGTCAAGCC	Reverse outer (for 7427)
RU-O-24308	TGACTGGTGAGTACTCAACCAAG	Forward outer (for 7427)
Generation of E mutations in pZIKV/HPF/CprM*E*		
RU-O-24998	AGGAGTCGGGGCGAAGAAGATCAC	E393A forward
RU-O-24999	GTGATCTTCTTCGCCCCGACTCCT	E393A reverse
RU-O-25000	AGGAGTCGGGGAGGCGAAGATCAC	K394A forward
RU-O-25001	GTGATCTTCGCCTCCCCGACTCCT	K394A reverse
RU-O-24379	ACTTGGTCATGATACTGCTGATTGCCCCGGCATACAGCATCAGGTGCATAGGAGT	Forward outer
RU-O-24380	TTCGAACCGCGGCTGGGTCCTATTAAGCAGAGACAGCTGTGGATAAGAAGATC	Reverse outer
Generation of pDENV1/PUO-359/CprME		
RU-O-24611	CTTGACCGACAATTGCATGAAG	Upstream of SnaBI site forward
RU-O-24610	GTCTTTTTCCGTTGGTTGTTCATAGCCTGCTTTTTTGTACAAAC	CMV promoter-Capsid fusion reverse
RU-O-24608	GTTTGTACAAAAAAGCAGGCTATGAACAACCAACGGAAAAAGAC	CMV promoter-Capsid fusion forward
RU-O-24609	TTCGAACCGCGGCTGGGTCCTATTACGCCTGAACCATGACTCCTAGGTAC	E C-terminus-SacII site reverse
Generation of pZIKV/HPF-CprM/MR766-E		
RU-O-24994	CAAAGAGTGGTTCCATGACATCCCATTG	BspHI site mutation forward
RU-O-24995	CAATGGGATGTCATGGAACCACTCTTTG	BspHI site mutation reverse
RU-O-24379	ACTTGGTCATGATACTGCTGATTGCCCCGGCATACAGCATCAGGTGCATAGGAGT	Forward outer
RU-O-24380	TTCGAACCGCGGCTGGGTCCTATTAAGCAGAGACAGCTGTGGATAAGAAGATC	Reverse outer

Table S5. Data collection and refinement statistics. Related to Figure 5.

	Z006–ZIKV EDIII	Z004–DENV1 EDIII
Data Collection		
Resolution Range (Å)	29.75 - 3.0 (3.107 - 3.0)	37.11 - 3.0 (3.107 - 3.0)
Space group	R 3 2 :H	P 4_3 2_1 2
Cell dimensions		
a, b, c (Å)	385.08, 385.08, 56.64	74.23, 74.23, 190.76
α, β, γ (º)	90, 90, 120	90, 90, 90
Total reflections	170795 (25781)	121867 (20556)
Unique reflections	31520 (3151)	11154 (1101)
Multiplicity	5.4 (5.6)	10.9 (11.6)
Completeness (%)	98.71 (99.78)	98.30 (99.73)
Mean I/s(I)	8.2 (2.3)	11.1 (3.3)
Wilson B-factor (Å^2)	77.8	58.7
R_{merge}	0.120 (0.598)	0.179 (0.829)
R_{pim}	0.083 (0.410)	0.079 (0.358)
CC1/2	0.992 (0.779)	0.994 (0.869)
Refinement		
R_{work}/R_{free}	0.212/ 0.257	0.236/ 0.281
Number of atoms	7905	3904
Macromolecules	7892	3904
Ligand	13	0
Protein residues	1051	513
RMS (bonds) (Å)	0.007	0.003
RMS (angles) (˚)	0.85	0.64
Ramachandran favored (%)	90.8	93.0
Ramachandran allowed (%)	7.6	6.8
Ramachandran outliers (%)	1.6	0.2
Rotamer outliers (%)	8.8	0.9
Clashscore	9.2	2.5
Average B-factor (Å^2)	103.78	59.31
Number of TLS groups	6	17

Statistics for the highest-resolution shell are shown in parentheses.

Table S6. Antibody-antigen contacts. Related to Figure 5.

Contacts are defined as residues in which any atom is within 4 Å of an atom from a residue on the interacting partner using AntibodyDatabase (West et al., 2013). The table is organized by antibody residue, listing all antigen residues contacted by each antibody residue (ordered by contact distance). Antibody residues are highlighted when corresponding interactions occur in both complexes. For the highlighted interactions additional information is listed including the antibody residue's origin in V(D)J recombination and the residue distribution at that antibody position in the sequenced antibody clones.

Chain	Z006 Fab	ZIKV EDIII	Closest distance	Z004 Fab	DENV1 EDIII	Closest distance	Description	Origin	Count in clones
HC	N31	Q350, T351							
	Y52	T351							
				I53	P336, A382				
				D54	M301				
	E55	K340, S306, L307, Y305							
	S56	L307		S56	M301, V300				
	T57	S306							
	Y58	L307, S306, T309	3.07	Y58	M301, V300, T303	2.54	Tyr-OH w/ antigen backbone oxygen	V Germline	68 Y, 1 W
	R96	E393	3.44	R96	E384	2.55	electrostatic/salt bridge	N addition	68 R, 1 Q
	S97	K395, G392, E393							
	N98	L352, G392, V391, K395							
	G99	G392		R99	G383, S338, A382				
	W100	T309, K394		G100	G383				
	S100A	G392		V100A	T303				
	S100B	E393							
				E100C	E384, G383, K385, A382				
				L100D	E384				
LC	Q27	T335	3.28	Q27	T329	3.96		V Germline	58 Q, 6 H, 5 R
				S30	E327				
	W32	K394, A311	3.08	W32	K385, S305, E327	3.37	hydrophobic (plus cation-pi)	V Germline	68 W, 1 L
	Y49	E393							
	Q50	E393							
	Y91	K394, E393	2.45	F91	K385	2.28	hydrophobic (plus Tyr-OH/Glu H-bond for ZIKV)	V Germline/SHM	57 Y, 12 F
	S92	A310, K394, T309	3.03	Y92	G328, K385, 327, G304, T303				
	T93	T335, G334, D336, A310, T309	3.22	S93	T329, T303, G328	2.68	H-bond to antigen backbone nitrogen	V Germline/SHM	42 S, 23 T, 3 N, 1 G
	F94	T309, D336	3.42	V94	T303, T329, D330	3.1	antigen sidechain H-bond to LC backbone plus vDW for antibody sidechain		43 Y, 13 F, 12 V, 1 S
				W96	T303				